

## **Efficiency of copper and cupronickel substratum to resist development of diatom biofilms**

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### **Abstract**

This study on the population dynamics of fouling diatoms from a biofouling rich monsoon influenced tropical bay revealed that irrespective of the season and exposure period, the highest diatom abundance and species were encountered on non-toxic (fiberglass>glass) compared to toxic (copper–Cu and cupro-nickel–Cu-Ni) substrata. Though the diatom recruitment on toxic substrata is noticed within 24h, the multiplication of the settled diatoms was not significant, even with an increase in the exposure period (days to weeks). The diatoms recorded on toxic substrata are the result of fresh recruitment. Results indicated that the potential of Cu and Cu-Ni panels to resist diatom fouling are the same. The dominance of raphid-pennate diatoms (*Navicula*, *Amphora*, *Nitzschia* and *Thalassionema*) throughout the year indicated them as ideal candidates for antifouling studies. The negligible contribution of araphid-pennate diatoms (*Licmophora* and *Grammatophora*) and the influence of tycho-pelagic/bloom forming diatoms (*Fragillariopsis* and *Skeletonema*) on fouling community are the other noteworthy observations. Concern with the adverse impact of Cu pollution on the ecosystem this study recommends the incorporation of Cu alloy (Cu-Ni) in the marine structures to combat biofouling as Cu-Ni has a slower leaching rate of the toxic product such as  $Cu_2O$ .

**Key words:** *Biofilms, Diatoms, Copper, Cupro-Nickel, Season, Exposure Period*

## Introduction

Biofouling of artificial structures is a serious problem in the marine environment resulting in vast economic losses for a number of industries (Townswain, 2003; Swain et al., 2007; Schultz et al., 2011; FitrIDGE et al., 2012). The term 'biofouling' refers to the undesired accumulation of micro-(eg. bacteria, cyanobacteria, diatoms) and macro-organisms on any artificial structure (Callow & Callow 2002). Among the micro-organisms, diatoms are of interest because of the following (i) facilitate development of marine biofouling (eg. Patil and Anil, 2005a; Cooksey et al., 2009) (ii) cause significant economic penalties (Schultz, 2004, 2007), environmental impacts (Corbett and Fishbank, 1997) and hinder the operation of optical sensors used for ocean monitoring and (iii) the diatoms have been reported to be highly resistant to biocidal antifouling coatings (Callow, 1986; Jelic-Mrcelic et al., 2006; Molino and Wetherbee, 2008; Molino et al., 2009; Zargiel et al., 2011; Briand et al., 2012) as well as found to be dominant on fouling release coatings (Cassé and Swain, 2006; Molino et al., 2009; Dobretsov and Thomason, 2011).

Historically, marine biofouling or biofilm control has been achieved by exploiting the toxicity of metals, biocides and organometals to the fouling organisms and incorporating them in paints/coatings (Swain et al., 2010). The ban of the most effective tributyl tin (TBT) based coatings to prevent biofouling from commercial ships led to the rapid increase in the application of alternate coatings (eg. copper based/fouling release coatings). Among the many alternatives, copper (Cu) and cupronickel (Cu-Ni) materials or cuprous oxide coatings are widely used as component in the marine structures because of their biofouling and corrosion resistance (Stanczak 2004). At present there are several reports that describe the diatoms colonization/communities on Cu based antifouling surfaces in terms of spatial (Callow, 1986; Molino et al., 2009; Zargiel et al., 2011), water quality (Briand et al., 2012) and the nature of immersion (Cassé and Swain, 2006). However, information on the biofilm diatom community structure is limited in terms of exposure periods during different climatically defined environmental conditions (Molino et al. 2009). In this study, Cu and Cu-Ni (70:30%) coupons, which are most popularly used material (Powell et al., 2000), was utilized to evaluate the biofouling diatom community dynamics in a monsoon influenced tropical bay located along the west coast of India facing the Arabian Sea. Further the findings from the simultaneous studies in the same region during the same period on the natural levels of diatom fouling of various non-toxic (both hydrophobic and hydrophilic) substrates (Patil and Anil, 2005b) is also considered for comparison. Biofouling potential is considerably high in the

study location, with macro-fouling setting in a few days' time (3-5 days). In view of this the results from this study will serve as a bench mark.

## **Materials and Methods**

### *Study area*

All the investigations were carried out at Dona Paula Bay, Goa, located at the mouth of the Zuari estuary, Goa, ( $15^{\circ} 27.5' N$ ,  $73^{\circ} 48' E$ ), on the west coast of India (Fig. 1). This estuary is classified as a tide-dominated, coastal, plain estuary. The river Zuari has its source in the Western Ghats, and it extends up to 70 km before meeting the Arabian Sea. This river is influenced by seawater inflow up to a considerable distance inland, and it receives a large quantity of fresh water from Zuari River and precipitation during the southwest monsoon season. Based on this, a year has been classified into three seasons viz. monsoon season (June–September), followed by a recovery period during the post-monsoon (October–January) and thereafter a stable pre-monsoon period (February–May).

In this study two separate experiments were conducted for the assessment of biofilm diatoms. In experiment 1 panels were deployed for short-term (for a period of 4 days) every month for a period of 14 months whereas in experiment 2 panels were deployed for long-term (for a period of 4 weeks) once in three different seasons. Hereafter experiment 1 and 2 will be referred as short-term and long-term experiments respectively. During short-term experiment, surface water samples (in triplicate) were collected every month for 5 consecutive days from the study site, for the following parameters: temperature, salinity, dissolved oxygen, dissolved nutrients ( $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$ ,  $\text{PO}_4\text{-P}$  and  $\text{SiO}_3$ ) and diatoms. Water temperature was recorded at the sampling site. Salinity (Mohr-Knudsen titration method), dissolved oxygen and nutrients ( $\text{NO}_2\text{-N}$ ,  $\text{NO}_3\text{-N}$ ,  $\text{PO}_4\text{-P}$  and  $\text{SiO}_3$ ) were analysed by following standard procedures (Parsons et al. 1984). Chlorophyll *a* was determined by 90% acetone extraction method using spectrophotometer (Shimadzu make). A known volume of the water sample was preserved with 4% Lugol's iodine solution for estimating the diatom population ( $\text{cells l}^{-1}$ ) microscopically.

### *Panel exposure*

The panels of even surface (admeasuring  $15 \times 10 \times 0.2$  cm) of commercial grade copper (Ni-0.05%, Cu-rest) and cupro-nickel 70/30 (Fe-0.5%, Ni-26.8%, Cu-rest) with 8 mm holes drilled at one end were bolted to fiberglass flats with PVC nuts and bolts and suspended at the sub-surface level (~1m below

lowest low tide level). Earlier reports (Mitbavkar and Anil, 2000) and personal observations of the present study area have revealed the dominance of micro-organisms on substrates for one to four days, followed by the arrival of macro-organisms especially on a variety of non-toxic surfaces. Keeping this in view, panels were exposed for four consecutive days, once every month in order to obtain 1, 2, 3 and 4 days old microfilm over a period of 14 months (November '98 to January 2000). The data from the Cu and Cu-Ni panels are presented. The published data from non-toxic surfaces (Patil and Anil, 2005b) for the same period and study area are considered for comparison. Since we did not encounter macro-organisms till 4<sup>th</sup> day on the Cu and Cu-Ni coupons, a long-term exposure of these panels was undertaken. In long-term experiment (experiment 2), the exposure periods were four weeks, once during pre-monsoon, monsoon and post-monsoon season of the year 1999 in order to obtain 1, 2, 3 and 4 weeks old biofilm. In this experiment non-toxic panels were not exposed because the macro-fouling which starts after 5 days of exposure and thereby not possible for comparison. The results are presented in terms of diatom abundance per decimeter square ( $\text{dm}^2$ ). Prior to deployment in both the experiments all the test panels were cleaned with 20% (V/V) hydrochloric acid followed by tap water and distilled water in succession. The panels were then dried at 40<sup>o</sup> C for 2 hours. They were subsequently sealed into airtight polythene bags till the time of deployment.

#### *Assessment of diatom settlement*

Panels (in replicates of 5) were removed every consecutive day till the fourth day during short term deployments and every consecutive week till the fourth week during long term deployments. The retrieved panels were then scraped with a nylon brush (Patil and Anil, 2005c) into known quantities (~100 ml) of 0.45  $\mu\text{m}$  membrane filtered seawater. The scraped material was then fixed with 4% Lugol's iodine. This preserved material was then concentrated by subjecting to sedimentation in a settling chamber. The diatom flora of the concentrated samples were enumerated both qualitatively and quantitatively using a compound microscope (Olympus make). Diatoms were identified using standard identification keys and cell abundance is expressed as cells per decimeter per square ( $\text{dm}^2$ ). Simultaneously, another set of coupons obtained during short-term exposure was used for chlorophyll *a* analysis. Further, in short term experiment the biofilm chlorophyll *a* from scraped material of additional panels (5 replicates) was also estimated. Chlorophyll *a* was determined by 90% acetone extraction method using spectrofluorometer (Shimadzu make).

### *Data analysis*

The fouling diatom communities were subjected to following univariate and multivariate analyses. Univariate techniques included the calculation of species count (S), Shannon-Wiener's diversity index (H'), species richness (d) and evenness (J') of the diatom community from the fouling film. *Multivariate analysis* included multiple analysis of variance (MANOVA) and clustering analysis. For MANOVA the fourth root-transformed data on the fouling diatom abundance was used to evaluate temporal (exposure period and months/seasons) and substratum variance. This analysis was performed with and without the data from non-toxic surfaces (fiberglass and glass). For clustering analysis, diatoms averaging more than 5% of the total diatom community was used with respect to species for days (1, 2, 3 and 4 days) and weeks (1, 2, 3 and 4 weeks) of submersion. Clustering was performed through Bray Curtis similarity and group average method. Data was subjected to fourth root transformation prior to analysis. Univariate and cluster analysis were performed using PRIMER software version 5. Additionally linear regression analysis was also performed on the log-transformed values of diatom abundance and chlorophyll *a* concentration of the fouling diatom community to evaluate their relationship obtained during short-term exposure.

### **Results**

#### *Environmental parameters of the surrounding water column*

Results of the environmental data (temperature, salinity, dissolved oxygen and dissolved inorganic nutrients) are published elsewhere (Patil and Anil 2009b). During the study period, the temperature ranged from 25 °C (February 1999) to 30°C (November 1998) and salinity from 15 to 35. The low salinity was observed during monsoon i.e. August 1999. Dissolved oxygen ranged from 3.0–6.1 ml (Figure 2b). Nitrate (NO<sub>3</sub>-N) peaks were observed in April, July and December 1999. Nitrite (NO<sub>2</sub>-N) peaked in March 1999 and phosphate (PO<sub>4</sub>-P) in May 1999. Silicate (SiO<sub>3</sub>) peaked in July 1999. The low salinity and high nutrients observed during monsoon in due to the freshwater influx from Zuari River and rainfall.

#### *Diatoms in 1 to 4 day old microfilms*

A total of 54 [(45 pennates and 9 centrics) belonging to 28 genera (21 pennates and 7 centrics)] and 48 diatom species [(40 pennates and 8 centrics) belonging to 25 genera (18 pennates and 7 centrics)] were encountered on both Cu and Cu-Ni respectively (Table 1). The pennate/centric diatom percentage

ratio on both the substratum remained above 1 on all occasions indicating the dominance of pennate diatoms (Supplement Fig. 1).

The temporal variation in the fouling diatom counts, abundance, diversity, evenness and species richness for both the panels revealed that the diatom abundance and species count were minimum during monsoon season (Fig. 2). Diatom abundance almost remained constant with the exposure period. Diversity and richness followed a similar pattern. Species count also showed a decreasing trend with an increase in exposure. Diatom evenness remained constant with an exposure period, indicating that the diatoms are evenly distributed. In case of copper, the ranges for diversity, species richness and evenness for day 1 are 1.87 to 3.76, 0.75 to 3.08 and 0.7 to 0.94 respectively whereas on day 4 the ranges were 2.64 to 3.67, 1.42 to 2.98 and 0.75 to 0.93 respectively. In the case of cupronickel, the ranges for diversity, species richness and evenness for day 1 are 2.25 to 3.36, 0.87 to 3.15 and 0.75 to 0.94 respectively whereas on day 4 the ranges were 2.45 to 3.39, 1.10 to 4.22 and 0.65 to 0.95 respectively. Linear regression analysis between the total diatom abundance and chlorophyll *a* concentrations showed a significant relationship only for initial days i.e. up to 2 days (Fig. 3a and b).

Cluster analyses of the species for all days revealed that in the case of Cu (6 species - day 1 and 2, 11 species - day 3 and 4) and Cu-Ni (7, 9, 5 and 8 species for days 1, 2, 3 and 4 respectively) species belonging to cluster I dominated the respective day old microfilm community (Supplement Figs. 2 and 3). Among the species belonging to these clusters, *N. transitans* var. *derasa* f. *delicatula* was the most dominant, followed by *Amphoracoffeaeformis*, *A. rostrata*, *Thalassionemanitzschioides*, *Cylindrothecaclosterium*, *Cocconeiscutellum*, *Grammatophora marina* and *Nitzschia sigma*. The remaining species of the cluster formed the next dominant forms. Among the ungrouped individuals, *Fragillariopsis* sp. contributed significantly to the microfilm community [about 20% during the monsoon for day 1 and 2 (Table 2a-b)] whereas the rest of the clusters or individual units for all exposure days formed the minor component.

#### *Diatoms in 1 to 4 weeks old microfilms*

A total of 28 [(21 pennates and 1 centrics) belonging to 16 genera (10 pennates and 6 centrics)] and 31 diatom species [(25 pennates and 5 centrics) belonging to 18 genera (13 pennates and 5 centrics)] were encountered on both Cu and Cu-Ni respectively (Table 1). Similar to short term exposure, the pennate/centric diatom percentage ratio on both the substratum remained above 1 on all occasions indicating the dominance of pennate diatoms (Supplement Fig. 1). The fouling diatom abundance and all

the univariate measures were minimal during the monsoon ( $H' - 2.42 \pm 0.3$ ;  $d - 1.18 \pm 0.5$  and  $J' - 0.84 \pm 0.1$ ) compared to the pre-monsoon ( $H' - 2.42 \pm 0.3$ ;  $d - 1.18 \pm 0.5$  and  $J' - 0.84 \pm 0.1$ ) and post-monsoon ( $H' - 2.42 \pm 0.3$ ;  $d - 1.18 \pm 0.5$  and  $J' - 0.84 \pm 0.1$ ) period (Fig. 4). In case of copper, the average values of univariate measures for different seasons are as follows: (i) during monsoon  $H'$ ,  $d$  and  $J'$  were  $2.42 \pm 0.3$ ,  $1.18 \pm 0.5$  and  $0.84 \pm 0.1$  respectively; (ii) during post-monsoon  $H'$ ,  $d$  and  $J'$  were  $3.2 \pm 0.3$ ,  $1.91 \pm 0.4$  and  $0.90 \pm 0.1$  respectively and (iii) during pre-monsoon  $H'$ ,  $d$  and  $J'$  were  $2.62 \pm 0.6$ ,  $1.43 \pm 0.5$  and  $0.82 \pm 0.1$ . In case of Cu-Ni, the average values of univariate measures for different seasons are as follows: (i) during monsoon  $H'$ ,  $d$  and  $J'$  were  $2.53 \pm 0.2$ ,  $1.04 \pm 0.3$  and  $0.95 \pm 0.1$  respectively; (ii) during post-monsoon  $H'$ ,  $d$  and  $J'$  were  $2.92 \pm 0.1$ ,  $1.31 \pm 0.3$  and  $0.96 \pm 0.1$  respectively and (iii) during pre-monsoon  $H'$ ,  $d$  and  $J'$  were  $2.99 \pm 0.6$ ,  $1.79 \pm 1.5$  and  $0.92 \pm 0.1$ .

However, MANOVA of diatom abundance, species count, diversity, evenness and richness showed insignificant variations between the seasons, exposure period and substrata (Supplementary table 3). During all the seasons the diatom population was evenly distributed ( $J' > 0.82$ ) with exposure (Fig. 4). Cluster analysis of the sampling periods based on diatom abundance at 40% similarity level revealed one group with one ungrouped individual sampling period for both Cu and Cu-Ni substrata (all weeks) (Supplement Fig. 4). Cluster I comprised of 2 sampling seasons (pre- and post-monsoon) whereas monsoon formed a separate individual unit. The ungrouped sampling period is characterized by low diatom population compared to other sampling periods.

The results of species-wise cluster analysis for Cu and Cu-Ni for all weeks at 50% similarity level revealed that the number of species decreased with exposure period (Supplement Fig. 5 and 6). In both the panels, the clusters comprising the following raphid pennate diatoms *N. transitans* var. *derasa* f. *delicatula*, *T. nitzschoides*, *A. coffeaeformis*, *N. sigma*, *Cylindrotheca closterium*, *N. subinflata* formed the dominant group and were present for most part of the study period, whereas the rest of the species formed the minor components. On certain occasions the contribution of certain tycho-pelagic diatoms such as *Melosira nummuloides* in Cu and bloom forming diatoms (*Skeletonema costatum* and *Fragillariopsis*) in Cu-Ni was also clearly evident. However, such a kind of influence was not observed during short-term exposure. Another noteworthy observation was the absence/negligible contribution of some raphid (*Achnanthes* and *Cocconeis*) and araphid pennate diatoms (*Grammatophora* and *Licmophora*) compared to those found on non-toxic materials.

## Discussion

### *Comparison of variations in diatom communities from toxic and non-toxic surfaces*

Irrespective of the season and exposure period, the highest diatom abundance and species were encountered on non-toxic substrata (fiberglass followed by the glass) compared to toxic (Cu and Cu-Ni) substrata (Table 1; Supplement Fig. 7). Such substratum preferences indicate either an active choice by the diatoms, or that the physical and chemical conditions of the substrata encourage or discourage settlement or entrapment of some species. While selective community development on toxic surfaces is well known (Daniel and Chamberlain, 1981), any differences in communities developing on non-toxic surfaces are due to more subtle environmental influences, for example, topography, surface charge, surface tension or nutrient availability (Fletcher, 1980; Escher and Characklis, 1980; Fattom and Shilo, 1984; Becker and Wahl, 1991; Sekar et al., 2003). Although the diatom communities are similar, most of the diatom species showed significant substratum preferences (Table 1). These substratum variations, which resulted in higher diatom recruitment on non-toxic substrata was also responsible for the significant relationship in diatom abundance and chlorophyll *a* concentration for non-toxic surfaces (Patil and Anil, 2005b; Mitbavkar and Anil, 2008), while it was restricted up to 2 days-old community on toxic substrata (Fig. 2), indicating that diatoms are the major contributors among the autotrophs in the microfilm. In the case of toxic panels, the non-significant relation between diatom abundance and chlorophyll *a* on day 3 and 4 (Fig. 2) can be attributed (i) to the toxic effect on microfilm diatoms and (ii) the non-inclusion of the contribution of the pico-phytoplankton groups (*Prochlorococcus*-like organisms, *Synechococcus* and the picoeukaryotes), which recently reported their dominance in biofilms from the study region (Mitbavkar et al., 2012). Literature also points out that the copper exposure caused stronger effects on periphyticalgal biomass and community structure (Guasch et al. 2002), pico-phytoplankton community (Debelius et al., 2010) as well as fouling assemblage diversity (Canning-Clode et al., 2011). However, further specific studies in this direction using biofouling organisms are essential.

The fouling population on Cu (30 pennates and 7 centrics) and Cu-Ni (35 pennates and 5 centrics) coupons revealed the dominance of pennate diatoms in terms of abundance and number of species. *Amphora*, *Cocconeis*, *Licmophora*, *Navicula*, *Nitzschia*, *Pleurosigma*, *Synedra* and *Thalassionema* were the dominant pennate diatoms (Table 2). However, their percentage composition varied depending on the exposure period and other coexisting species. For example, with increase in



exposure period *Thalassionemanitzschoides* dominated the biofilm community from Cu coupons whereas the dominance of *Amphora*, *Nitzschia* and *Thalassionema* was observed on Cu-Ni panels. Irrespective of exposure period, the contribution of *Navicula* (*N. transitans* var. *derasa* f. *delicatula*) to the biofilm community remained constant. On non-toxic substratum the dominant diatoms [*N. transitans* var. *derasa* f. *delicatula* (2-5 fold), *Amphora* spp. (2-10 fold), *Nitzschia* spp. (upto 25 fold), *Thalassionema* spp. (upto 4-fold)] showed many-fold increase on day 4 (Patil and Anil, 2005). Such significant increment in the diatom population was not observed on Cu and Cu-Ni panels. The very slow rate of diatom slime formation on toxic panels could be either due to (i) dissolution or leaching from the panels, (ii) the toxic nature of the substratum (Chamberlain and Garner, 1988; French and Evans, 1988) and (iii) different sensitivities of diatoms towards toxic panels (Molino et al., 2009). Earlier study by Sawant et al. (1995) on corrosion and microfouling of Cu and Cu-Ni panels (similar to those used in the present study) from tropical marine waters indicate that corrosion rate is three times less in Cu-Ni (4.2 to 5.0 mg dm<sup>-2</sup>d<sup>-1</sup>) than Cu (11 to 15.4 mg dm<sup>-2</sup> d<sup>-1</sup>) and corrosion rate varied seasonally i.e. maximum during pre-monsoon and least during monsoon. They also showed that the microfouling assemblage showed an inverse relationship with corrosion rate. X-ray diffraction analysis of the corrosion products revealed Cu<sub>2</sub>O as the major component, which is known to be toxic to the settled organisms (Sawant et al., 1995).

#### *Temporal variations in diatom communities from toxic surfaces*

Earlier studies on the natural levels of diatom fouling from a variety of surfaces indicate less fouling during monsoon compared to non-monsoon seasons and is attributed low diatom numbers in the surrounding water column as well as monsoon driven disturbances such as high wave action, low light (cloud and high turbidity) and salinity variations due to heavy land runoff, and precipitation (Patil and Anil, 2005b). A similar kind of seasonal variations in the diatom fouling is also observed with respect to Cu and Cu-Ni in both short and long term studies. However, ANOVA of cell abundance showed an insignificant variation between the sampling periods, whereas the species composition, diversity, evenness and species richness showed significant variations with respect to sampling periods. These findings were same for both short and long term exposure experiments. These results clearly demonstrate that the potential of the tested materials to resist diatom fouling are same irrespective of exposure periods, seasons and the presence of the type of organisms/community in the ambient water column.

Further the persistence of *N. transitans* var. *derasa* f. *delicatula*, *Amphora*, *Nitzschia* and *Thalassionema* throughout the investigation period further reveals their tolerance towards Cu and Cu-Ni and also the recruitment process is continued round the year. These species are common fouling diatoms and also have the potential to colonize surfaces irrespective of the nature of immersions i.e. static and dynamic (Zargeil and Swain 2014). Another noteworthy observation was that the influence of tycho-pelagic diatoms (temporarily dislodged benthic diatom community) such as *Melosira nummuloides* and bloom forming diatoms (*Skeletonema* and *Fragillariopsis* sp.) was observed to exert its influence significantly (pennate and centric ratio <1) on the microfilm community from non-toxic surfaces (Patil and Anil, 2005b). Such a kind of influence was also seen in the cases of Cu and Cu-Ni substrata, though not significant.

#### *Effect of exposure period*

Generally, in the study region, the recruitment of diatoms on non-toxic surfaces (eg. fiberglass, glass) take place within 24h of its exposure to marine environments and within few days of prolonged exposure several fold increase in the population is noticed due to continuous recruitment from the surrounding waters and growth of already settled diatoms (Patil & Anil 2005). From day four or five, macrofouling organisms, especially marine invertebrates (eg. barnacles, bryozones, hydroids) start arriving on to the surfaces which results into the development of mature macro-fouling community after an exposure period of several weeks to months). In the present study, though the recruitment of diatoms on Cu and Cu-Ni is noticed within 24h the significant growth of the settled diatoms is not observed as seen in non-toxic materials even with an increase in the exposure period (i.e. from days to several weeks). In both short- and long-term exposures, the biofilm diatom community showed insignificant variation among the Cu and Cu-Ni indicating that the characteristics (i.e. dissolution or leaching) of both the surfaces towards the resistance of the development of biofilm is same. In short-term exposure, biofilm diatom abundance and diversity increased or remained constant with exposure period, whereas the long-term exposure did not show any considerable increase in diatom abundance with exposure period. However, the species diversity decreased compared to that during short-term exposure. ANOVA of cell abundance showed an insignificant variation between the exposure periods, whereas the species composition, diversity, evenness and species richness showed significant variations with respect to exposure periods. These results clearly demonstrate that the diatoms recorded at different exposure periods is the result of recruitment and not the growth of the recruited diatoms as it is generally seen on non-toxic surfaces. The dominance of pennate diatoms such as *Navicula*, *Amphora*, *Nitzschia*, and

*Thalassionema* during both short and long term exposure for most part of the year indicated their potential to foul or recruit the tested substrata. The presence of *Amphora*, *Navicula* and *Nitzschia* in considerable numbers on a range of antifouling paints or coatings containing copper is well reported (Callow 1986, 1986; Cassé and Swain 2006; Pelletier et al. 2009; Briand et al. 2012; Zargiel and Swain 2014, Hunsucker et al. 2014, Muthukrishnan et al. 2014). The noteworthy observations here are the absence of *Achnanthes* during long-term deployment and the prevalence of *Thalassionema* on copper substratum on most occasions. Although the mechanism of attachment of *Amphora* and *Achnanthes* to toxic surfaces is known (eg. Hunsucker et al. 2014 and the references therein) further studies to unravel the settlement mechanism of other diatoms belonging to the genera *Navicula*, *Nitzschia*, *Thalassionema* to toxic surfaces needs attention. It has been reported that the exopolysaccharides (EPS) produced by algae are negatively charged and rich in uronic acids exhibit high copper binding capacity (Kaplan et al. 1988). If this is so, then the fouling diatoms, which produce uronic acid containing EPS during biofilm formation (Bhosle et al. 1995) may offer some selective advantage to the diatoms either to attach or grow on copper surfaces. Previous studies have reported that the amount of exopolysaccharides and uronic acid production is different for *Amphora* and *Navicula* (Bhosle et al. 1995; Khandeparker and Bhosle 2000, Patil and Anil 2005c; Kodse and Bhosle 2010). This suggests that the diatom attachment to copper surfaces is also dependent on the amount of uronic acid production. However this aspect needs further investigation. Further Zargeil and Swain (2014) experiments confirm the differences in diatoms fouling (abundance and composition) between the static and dynamic immersion treatments. Hence dynamic immersion evaluation (horizontal shear stress and water pressure at different depths) also needs to be considered to understand the diatom adhesion and growth on to such toxic surfaces.

## **Conclusions**

We conclude that the Cu and Cu-Ni coupons are susceptible to diatom colonization. However the growth of colonized diatoms is significantly slow compared to non-toxic substrates. This trend was observed irrespective of the seasons, exposure periods and the presence of type of organisms. In both short-term and long-term exposures, with respect to exposure period, only diatom abundance showed insignificant variations while the species composition and other biodiversity measures showed significant variations. The diatoms recorded during different exposure periods are the result of new recruitment and not the multiplication of the already recruited diatoms. The dominance of pennate diatoms such as *Navicula*, *Amphora*, *Nitzschia*, and *Thalassionema* throughout the study period indicated their potential to tolerate the toxicity of the substrata. It was also observed that the potential of

the tested materials to resist development of diatom fouling were same. Concern with the adverse impact of Cu pollution on the ecosystem this study recommends the incorporation of Cu alloy (Cu-Ni) in the marine structures to combat biofouling as Cu-Ni has a slower rate of leaching of the toxic product such as Cu<sub>2</sub>O.

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Table I. Diatoms recorded from the surrounding water column and biofilm (both short and long term) developed on different substrata during the study.

Sl. No.	Taxon	Abbrev.	Short term					Long term	
			WC	Fg	Gl	Cu	CN	Cu	CN
<b>Pennates</b>									
1	<i>Achnanthes brevipes</i> Agardh	Ac. br		+	+	+	+		
2	<i>A. longipes</i> Agardh	Ah. lo		+	+	+	+		
3	<i>A. subsessilis</i> Kützing	Ac. su		+	+				
4	<i>Amphiprora gigantea</i>	Am. gi		+	+				
5	<i>Amphora coffeaeformis</i> (Ag.) Kützing	A. co	+	+	+	+	+	+	+
6	<i>A. hyalina</i> Kützing	A. hy		+	+	+	+	+	+
7	<i>A. ovalis</i> (Kützing) Kützing	A. ov		+	+		+		
8	<i>A. rostrata</i> Wm. Smith	A. ro		+	+	+	+	+	+
9	<i>A. turgida</i> Gregory	A. tu		+	+	+	+		
10	<i>Asterionellopsis glacialis</i> (Castracane)	As. gl	+						
11	<i>Bacillaria paxillifera</i> (O. F. Müller)	Ba. pa		+			+	+	
12	<i>Cocconeis scutellum</i> Ehrenberg	Co. sc	+	+	+	+	+	+	+
13	<i>Cocconeis</i> sp.	Co. sp		+	+	+	+	+	
14	<i>Cylindrotheca closterium</i> (Ehrenberg) Smith	Ci. cl	+	+	+	+	+	+	+
15	<i>Cymbella gastroides</i> Kützing	Cy. ga	+	+	+	+	+		+
16	<i>Diploneis smithii</i> (de Brebisson) Wm. Smith	D. sp		+	+	+	+		
17	<i>Fragilariopsis oceanica</i> (Cleve) Hasle	F. oc	+	+	+	+	+		+
18	<i>Grammatophora marina</i> (Lyngbye) Kützing	Gr. ma	+	+	+	+	+		+
19	<i>G. serpentina</i> Ehrenberg	Gr. se		+	+	+	+	+	
20	<i>Gyrosigma</i> sp.	Gy. sp		+			+		
21	<i>Hantzschia</i> sp.	Hn. sp		+	+	+	+		+
22	<i>Licmophora flabellata</i> (Greville) Agardh	L. fl		+	+	+			+
23	<i>L. gracilis</i> (Ehrenberg) Grunow	L. gr		+	+	+	+		+
24	<i>L. juergensii</i> Agardh	L. je		+	+	+			+
25	<i>L. paradoxa</i> (Lyngbye) Agardh	L. pa	+	+	+	+	+		+
26	<i>Meuniera membranacea</i> (Cleve) P. C. Silva comb. nov	N. me		+	+		+		
27	<i>Navicula clavicularis</i>	N. cl		+	+		+		
28	<i>N. crucicula</i> (Wm. Smith) Donkin	N. cr		+	+	+	+	+	
29	<i>N. granulata</i>	N. gr		+	+	+	+	+	
30	<i>N. subinflata</i> Grunow	N. su	+	+	+	+	+	+	+
31	<i>N. transitans</i> var. <i>derasa</i> f. <i>delicatula</i> Heimdal	N. de	+	+	+	+	+	+	+
32	<i>Navicula</i> sp. 1	N. sp1	+	+	+	+	+	+	
33	<i>Navicula</i> sp. 2	N. sp2		+	+	+			+
34	<i>Navicula</i> sp. 3	N. sp3		+	+	+	+		+
35	<i>Navicula</i> sp. 4	N. sp4		+	+	+	+		
36	<i>Nitzschia acicularis</i>	Ni. ac		+	+				
37	<i>N. angularis</i> Wm. Smith	Ni. an	+	+	+	+	+		
38	<i>N. bilobata</i> Wm. Smith	Ni. bi	+	+	+	+	+	+	+
39	<i>N. longissima</i> Ralfs in Pritchard.	Ni. lo	+	+	+	+	+	+	+
40	<i>N. panduriformis</i> Gregory	Ni. pa	+	+	+	+	+		+
41	<i>N. sigma</i> (Kützing) Wm. Smith	Ni. si	+	+	+	+	+	+	+
42	<i>Pinnularia rectangulata</i> (Gregory) Rabenhorst	Pi. sp		+	+			+	
43	<i>Pleurosigma angulatum</i> Sensu W. Smith	Pl. an	+	+	+	+	+	+	+
44	<i>P. elongatum</i> WM Smith	Pl. el	+	+	+	+	+		
45	<i>Pleurosigma</i> sp.	Pl. sp		+		+			
46	<i>Pseudo-nitzschia seriata</i> (Cleve) H. Pergallo	Pn. se	+	+	+	+	+	+	
47	<i>Pseudo-nitzschia</i> sp.	Pn. sp		+	+				
48	<i>Rhabdonema</i> sp.	Rd. sp		+					
49	<i>Stauroneis</i> sp.	St. sp		+					
50	<i>Surirella ovalis</i>	Su. ov	+	+	+	+			
51	<i>Surirella</i> sp.	Su. sp	+	+	+				
52	<i>Synedra affinis</i> Kützing	Sy. af		+	+	+	+	+	+
53	<i>S. gallioni</i> (Bory) Ehrenberg	Sy. ga	+	+	+	+	+	+	+
54	<i>Synedra</i> sp.	Sy. sp		+	+				
55	<i>Tabellaria</i> sp.	Tb. sp		+					



Table I (Continued)

Sl. No.	Taxon	Abbrev.	Short term					Long term	
			WC	Fg	Gl	Cu	CN	Cu	CN
<b>Pennates</b>									
56	<i>Thalassionema frauenfeldii</i> (Grunow) Hallegraeff	Th. fr		+	+	+	+		+
57	<i>T. nitzschoides</i> (Grunow) Mereschkowsky	Th. nit	+	+	+	+	+	+	+
58	<i>Thalassiothrix longissima</i> Cleve and Grunow	Th. lo		+	+	+	+		
<b>Centrales</b>									
1	<i>Asteromphalus</i> sp.	Ast. ma		+					
2	<i>Bacteriastrum hyalinum</i> Lauder	Ba. hy	+						
3	<i>Biddulphia pulchella</i> Gray	Bd. pu		+	+	+			
4	<i>B. rhombus</i> (Ehrenberg) Smith, W.	Bd. rh		+	+	+	+		+
5	<i>Cerataulina pelagica</i> (Cleve) Hendy	Ce. pe	+						
6	<i>Chaetoceros curvisetus</i> Cleve	Ch. cu	+	+	+	+	+	+	
7	<i>C. diversus</i> Ehrenberg	Ch. dv	+						
8	<i>C. lorenzianus</i> Grunow	Ch. lr	+						
9	<i>Climacodium frauenfeldianum</i> Grunow	Cl. fr	+						
10	<i>Corethron criophilum</i> Castracane	Cr. cr	+						
11	<i>Coscinodiscus marginatus</i> Ehrenberg	C. ma	+	+	+	+	+	+	
12	<i>C. radiatus</i> Ehrenberg	C. rad		+	+	+	+	+	+
13	<i>Coscinodiscus</i> sp.	C. sp	+						
14	<i>Cyclotella</i> sp.	Cyc. sp		+	+	+	+	+	
15	<i>Ditylum brightwellii</i> (West) Grunow	Di. br	+						
16	<i>Eucampia zodiacus</i> Ehrenberg	Eu. zo	+						
17	<i>Guinardia flaccida</i> (Castracane) Pergallo	Gu. fl	+						
18	<i>G. striata</i> (Stolterfoth) Hasle com. Nov	Gu. st	+						
19	<i>Helicotheca tamesis</i> (Shrubssole) Ricard	He. ta	+						
20	<i>Hemiaulus sinensis</i> Greville	He. si	+						
21	<i>Lauderia annulata</i> Cleve	La. an	+						
22	<i>Leptocylindrus danicus</i> Cleve	Lp. da	+	+	+				
23	<i>Melosira nummuloides</i> C.A. Agardh	M. nu	+	+	+	+	+	+	+
24	<i>Odontella regia</i> (M. Schultze) Ostenfeld	Od. re	+						
25	<i>O. sinensis</i> (Greville) Grunow	Od. si	+						
26	<i>Paralia sulcata</i> (Ehrenberg) Cleve	Pa. su		+	+				
27	<i>Planktoniella sol</i> (Wallich) Schutt	Pk. so	+	+	+				
28	<i>Proboscia alata</i> (Brightwell) Sundstrom	Pr. al	+	+				+	
29	<i>Rhizosolenia stolterfothii</i> H. Pergallo	Ri. st	+	+					
30	<i>Skeletonema costatum</i> Greville (Cleve)	Sk. co	+			+	+		+
31	<i>Striatella</i> sp.	St. sp	+						
32	<i>Thalassiosira eccentrica</i> (Ehrenberg) Cleve	Th. ec	+	+	+	+	+	+	+
33	<i>T. subtilis</i> (Ostenfeld) Gran	Th. sb	+						
34	<i>Thalassiosira</i> sp.	Th. sp	+						

Table II. Diatom abundance (no. dm<sup>-2</sup>), those contributing >5%, recorded from biofilm developed on copper and cupro-nickel substratum during 1 to 4 day exposure periods.

a. 1 d-old-biofilm		Diatoms																
Mon	Sub	Ac	Am	Coc	Fra	Gr	Li	N.de	N.spp	Ni	Pl	Sy	Tha	PF	Bd	Cos	Mel	CF
N-98	Cu	0	51	76	32	0	0	76	0	28	0	0	19	9	0	0	0	19
	CN	7	14	28	0	0	0	87	7	54	0	0	28	7	0	0	0	14
D-98	Cu	16	66	16	0	0	0	89	0	57	0	0	64	0	0	0	0	0
	CN	0	80	35	0	7	0	88	35	28	0	14	28	0	7	0	0	7
J-99	Cu	0	21	28	0	0	0	34	0	34	0	21	14	0	7	0	0	7
	CN	0	9	0	0	0	0	47	32	41	0	19	19	0	0	0	0	9
F-99	Cu	21	21	14	0	40	0	157	0	41	21	0	7	0	0	0	0	0
	CN	35	35	14	0	54	0	93	0	14	21	0	7	0	0	0	0	0
M-99	Cu	0	54	22	0	11	21	80	11	56	6	6	32	21	0	21	0	0
	CN	0	75	16	0	32	21	127	22	51	11	22	32	21	0	11	0	0
A-99	Cu	0	55	6	0	0	0	86	11	6	0	17	11	0	0	0	0	0
	CN	9	47	9	0	0	9	38	19	9	0	19	9	0	9	0	0	0
My-99	Cu	6	33	0	0	16	0	43	17	11	6	11	22	6	17	0	0	0
	CN	6	22	0	0	16	0	43	11	0	0	11	11	6	17	0	0	0
Jy-99	Cu	0	11	0	0	11	0	27	6	23	6	6	11	0	0	0	0	0
	CN	0	16	0	0	16	0	32	6	28	6	6	16	0	0	0	0	0
Ag-99	Cu	0	0	0	49	0	0	90	0	48	0	0	6	0	0	11	0	0
	CN	0	0	0	97	0	0	65	0	73	0	8	33	0	0	0	0	0
S-99	Cu	0	27	7	27	0	0	110	0	14	0	14	140	0	0	0	0	0
	CN	0	34	0	54	0	0	61	21	94	0	21	40	0	0	0	0	0
O-99	Cu	0	0	0	0	0	63	5	27	0	20	0	0	67	8	0	0	0
	CN	15	19	15	0	15	0	61	15	64	0	15	125	0	0	0	0	8
N-99	Cu	0	38	38	0	19	0	57	38	38	0	19	66	0	0	0	0	28
	CN	19	38	38	28	0	0	131	28	66	0	47	38	0	0	0	56	19
D-99	Cu	0	9	19	0	0	0	75	19	57	0	19	66	0	0	0	0	0
	CN	7	28	41	0	0	7	131	14	56	7	14	67	14	0	14	0	0
J-00	Cu	0	21	11	0	11	11	64	21	11	11	0	32	0	0	0	0	11
	CN	8	16	8	0	3	0	76	13	16	2	10	8	5	0	0	0	0

b. 2 d-old-biofilm		Diatoms																
Mon	Sub	Ac	Am	Coc	Fra	Gr	Li	N.de	N.spp	Ni	Pl	Sy	Tha	PF	Bd	Cos	Mel	CF
N-98	Cu	11	54	11	0	11	0	140	43	54	21	32	43	0	0	0	0	11
	CN	0	28	28	0	0	0	81	0	42	0	42	42	0	0	0	0	14
D-98	Cu	0	35	0	28	0	0	107	28	0	0	35	74	0	0	0	0	0
	CN	28	14	47	28	41	0	73	28	81	0	35	80	40	14	14	0	14
J-99	Cu	0	35	28	0	0	0	54	7	34	0	21	14	7	7	0	0	7
	CN	0	21	21	0	0	0	54	0	11	0	0	21	0	0	0	0	0
F-99	Cu	21	21	14	0	40	0	119	0	48	28	0	7	0	0	0	7	0
	CN	27	40	14	0	54	0	107	0	21	28	0	14	0	0	0	0	0
M-99	Cu	0	74	27	0	14	28	107	14	69	14	7	48	28	0	27	0	0
	CN	0	95	21	0	40	28	154	28	63	14	28	40	28	0	0	0	0
A-99	Cu	0	60	11	0	0	0	96	28	11	0	17	11	0	0	0	0	0
	CN	0	32	0	0	0	0	21	0	21	0	11	21	0	0	0	0	0
My-99	Cu	7	42	0	0	21	0	54	14	7	7	14	21	7	21	0	0	0
	CN	7	28	0	0	28	0	54	7	0	0	7	14	14	28	0	0	0
Jy-99	Cu	0	14	0	0	14	0	35	7	28	7	7	14	0	0	0	0	0
	CN	0	25	0	0	16	0	65	8	49	0	0	25	0	0	0	0	0
Ag-99	Cu	0	8	0	0	0	0	32	0	49	0	0	25	0	0	0	0	0
	CN	0	0	0	107	0	0	54	0	43	0	0	64	0	0	0	0	32
S-99	Cu	8	25	0	0	0	0	81	16	25	0	0	105	0	0	0	0	0
	CN	8	55	0	33	0	15	68	7	52	0	23	56	0	0	0	0	0
O-99	Cu	10	58	0	118	0	0	135	20	102	0	0	80	20	0	0	0	0
	CN	9	55	0	0	8	0	130	15	120	0	24	54	24	0	0	0	18
N-99	Cu	0	75	32	0	0	0	150	11	129	0	0	54	0	0	0	11	11
	CN	0	54	0	0	0	0	120	0	40	0	14	54	14	0	0	0	54
D-99	Cu	7	7	21	0	0	0	42	0	81	0	7	7	0	0	0	0	0
	CN	21	42	28	53	7	0	146	0	42	0	28	167	14	0	0	0	14
J-00	Cu	0	9	19	0	0	0	66	9	9	19	9	38	0	0	0	0	0
	CN	13	23	10	0	15	10	97	15	45	8	19	40	0	0	5	0	0

Table II (Continued)

c. 3 d-old-biofilm

Mon	Sub	Diatoms																
		Ac	Am	Coc	Fra	Gr	Li	N.de	N.spp	Ni	Pl	Sy	Tha	PF	Bd	Cos	Mel	CF
N-98	Cu	0	42	14	0	7	0	54	14	14	0	7	21	0	0	0	0	21
	CN	27	40	14	27	0	0	160	28	0	0	40	28	0	0	0	0	40
D-98	Cu	14	21	7	0	7	0	105	14	53	0	14	119	7	7	0	0	0
	CN	0	56	27	0	7	0	71	14	40	0	0	74	7	0	28	7	14
J-99	Cu	0	0	11	0	21	0	32	21	32	0	0	54	0	0	0	0	11
	CN	0	21	21	0	14	0	40	14	0	7	0	14	7	0	0	0	0
F-99	Cu	35	35	14	0	54	0	181	0	53	28	0	14	0	0	7	7	0
	CN	14	41	0	0	28	0	114	0	21	14	0	21	0	0	0	0	0
M-99	Cu	0	103	37	0	19	38	150	19	95	19	9	66	38	0	37	0	0
	CN	0	117	19	0	0	0	87	8	27	0	0	23	0	0	0	8	0
A-99	Cu	0	25	0	0	0	0	49	8	16	0	16	8	8	0	0	16	0
	CN	0	21	0	0	0	0	11	0	0	21	0	21	0	0	23	11	0
My-99	Cu	11	68	0	0	32	0	87	21	11	11	21	32	11	32	0	0	0
	CN	11	27	0	0	32	0	64	16	0	0	11	27	17	11	0	0	0
Jy-99	Cu	0	33	0	0	33	0	65	8	33	8	16	41	0	0	0	0	0
	CN	7	21	0	0	21	0	81	21	56	0	27	7	0	0	0	0	0
Ag-99	Cu	0	17	0	0	0	0	59	6	38	0	0	22	0	0	0	0	0
	CN	0	0	0	64	0	0	33	6	16	0	0	44	0	0	0	0	27
S-99	Cu	0	16	0	0	0	0	144	49	49	33	0	128	0	0	33	16	0
	CN	20	81	0	0	0	38	98	0	15	0	45	140	0	0	0	0	0
O-99	Cu	18	73	4	0	12	12	273	73	318	9	44	186	24	0	13	0	0
	CN	15	95	0	0	0	0	159	28	134	5	20	71	4	0	8	0	0
N-99	Cu	7	21	14	0	0	0	54	7	7	0	21	81	0	0	0	0	7
	CN	25	109	27	0	0	0	218	54	81	0	14	64	0	27	0	0	58
D-99	Cu	0	42	7	0	0	0	48	14	55	0	20	35	0	0	0	0	0
	CN	0	16	8	0	0	0	57	0	16	0	8	49	0	0	8	33	8
J-00	Cu	11	43	21	0	21	0	75	32	21	0	21	54	0	0	0	0	0
	CN	6	8	9	0	10	0	104	3	33	1	15	48	0	0	0	0	0

d. 4 d-old-biofilm

Mon	Sub	Diatoms																
		Ac	Am	Coc	Fra	Gr	Li	N.de	N.spp	Ni	Pl	Sy	Tha	PF	Bd	Cos	Mel	CF
N-98	Cu	0	34	0	47	0	0	54	7	28	0	7	28	7	0	0	0	0
	CN	19	131	9	0	28	0	141	47	57	0	19	28	0	0	0	28	9
D-98	Cu	0	55	21	0	7	0	74	14	56	0	42	27	0	0	0	0	7
	CN	0	14	0	0	0	7	21	7	7	0	0	14	0	0	14	7	0
J-99	Cu	0	21	14	0	28	0	54	42	28	0	0	47	0	0	0	0	21
	CN	0	21	14	0	14	0	33	7	0	0	0	7	7	0	0	0	0
F-99	Cu	28	47	14	0	67	0	146	0	47	35	0	28	0	0	0	14	0
	CN	14	41	0	0	28	0	80	0	21	7	0	14	0	0	0	0	0
M-99	Cu	0	138	33	0	49	33	239	33	106	16	25	73	33	0	32	0	0
	CN	0	136	20	0	0	0	114	8	30	0	0	30	0	0	0	0	0
A-99	Cu	0	33	0	0	0	0	33	8	16	0	0	8	8	0	0	0	0
	CN	0	43	0	0	0	0	54	11	0	11	11	21	0	0	0	0	0
My-99	Cu	16	66	8	0	49	0	137	25	16	16	25	33	16	8	0	0	0
	CN	14	35	0	0	40	0	81	21	0	0	14	34	21	14	0	0	0
Jy-99	Cu	0	47	0	0	40	0	53	21	56	14	21	40	0	0	0	0	0
	CN	7	22	16	0	16	6	90	28	54	0	0	27	11	0	0	0	0
Ag-99	Cu	0	49	0	22	11	0	105	11	59	0	0	27	0	0	0	0	0
	CN	0	0	0	80	0	0	41	7	21	0	0	54	0	0	0	0	34
S-99	Cu	0	33	0	0	0	0	228	35	69	14	14	93	0	0	14	21	27
	CN	0	48	5	87	3	0	330	28	90	13	27	95	8	0	3	0	0
O-99	Cu	101	64	0	15	0	0	264	20	174	43	28	90	8	0	0	0	4
	CN	19	23	0	0	0	8	131	19	53	8	0	42	4	0	8	0	0
N-99	Cu	0	14	0	0	0	0	54	21	0	0	0	35	0	0	0	0	40
	CN	0	145	13	0	0	0	241	25	109	0	13	49	0	0	13	0	13
D-99	Cu	0	122	27	0	0	0	107	28	100	0	42	107	0	0	0	0	20
	CN	0	25	37	24	13	13	108	13	63	0	25	73	13	0	13	0	0
J-00	Cu	19	47	38	0	38	0	122	28	47	0	28	75	9	0	0	0	0
	CN	6	15	9	0	10	5	111	15	42	4	15	58	0	0	3	0	0

Table III. Diatom abundance (no. dm<sup>-2</sup>), those contributing >5%, recorded from biofilm developed on copper and cupro-nickel substratum during 1 to 4 weeks exposure periods.

Seasons	Substrata	Weeks	Species														
			<i>Am</i>	<i>Coc</i>	<i>Fra</i>	<i>Li</i>	<i>N.de</i>	<i>N.spp</i>	<i>Ni</i>	<i>Pl</i>	<i>Sy</i>	<i>Tha</i>	PF	<i>Bd</i>	<i>Cos</i>	<i>Mel</i>	CF
Pre-mon	Cu	1	43	33	0	0	87	11	65	0	54	43	11	0	0	54	22
		2	109	0	0	0	109	0	27	54	27	109	0	0	0	0	0
		3	33	0	0	0	77	17	28	0	38	143	0	0	0	0	0
		4	106	0	0	0	22	17	28	0	62	364	0	0	50	0	0
	CN	1	16	0	0	82	47	7	40	7	23	61	14	2	2	0	7
		2	25	0	0	0	50	50	50	0	25	76	25	0	25	25	0
		3	109	54	0	0	54	0	217	54	0	163	0	0	0	0	0
		4	109	54	0	0	54	54	54	0	0	54	0	0	0	0	0
Mon	Cu	1	19	0	0	0	112	28	28	28	0	130	0	0	37	19	0
		2	12	0	0	0	82	0	0	23	0	105	0	0	23	12	0
		3	30	0	0	0	40	0	0	20	0	61	0	0	0	0	30
		4	25	0	0	0	126	0	0	50	0	151	0	0	50	25	0
	CN	1	16	0	16	0	39	0	23	0	8	23	0	0	0	0	23
		2	19	0	19	0	47	0	28	0	9	28	0	0	0	0	28
		3	47	0	0	0	70	0	70	0	0	70	0	0	0	0	0
		4	54	0	0	0	82	0	54	0	27	82	0	0	0	0	0
Post-mon	Cu	1	27	19	0	0	50	12	35	0	23	47	4	0	0	0	12
		2	58	29	0	0	97	10	78	0	49	68	0	0	0	0	0
		3	68	27	0	0	95	14	54	0	68	95	0	0	0	0	0
		4	151	76	0	0	177	25	101	0	76	126	0	0	0	0	0
	CN	1	27	14	0	27	54	27	27	14	0	41	0	0	41	27	0
		2	19	0	0	19	47	19	19	0	0	37	0	0	28	19	0
		3	25	0	0	50	76	50	50	0	25	76	0	0	25	0	0
		4	109	0	0	0	109	54	109	0	0	109	0	0	0	0	0

## Legends to figures

**Fig. 1.** Geographical location of study area.

**Fig. 2.** Temporal variation in the univariate measures (species count, abundance in log scale, diversity, evenness and richness) of the biofilm diatom population on (a) copper and (b) cupro-nickel substrata for different exposure periods (1, 2, 3 and 4 d).

**Fig. 3.** Linear regression analysis between diatom abundance (log transformed) and chlorophyll *a* concentration on (a) copper and (b) cupro-nickel substratum for different exposure periods (Day 1; Day 2; Day 3 and Day 4).

**Fig. 4.** Seasonal variation in the univariate measures (species count, abundance in log scale, diversity, evenness and richness) of the fouling diatom population on copper and cupro-nickel substrata for different exposure periods (Week 1; Week 2; Week 3 and Week 4).

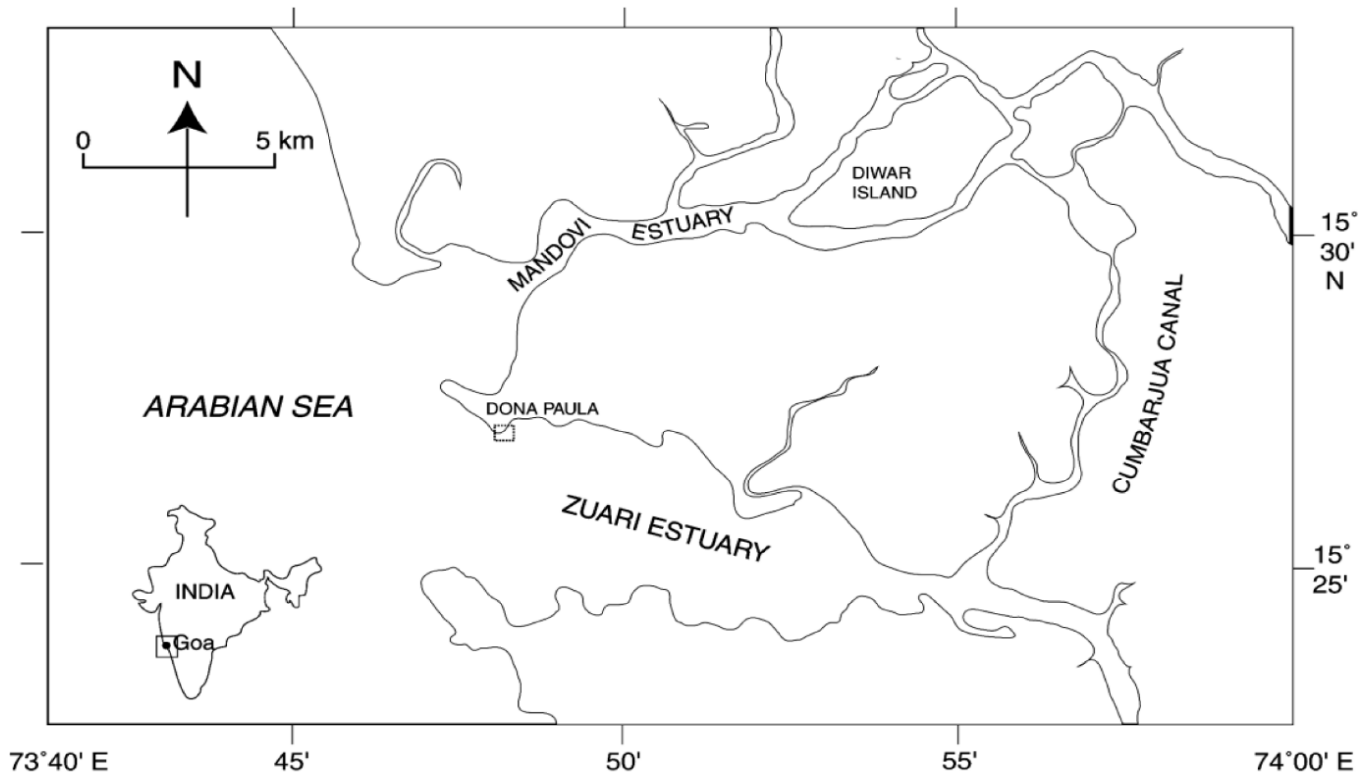


Fig. 1

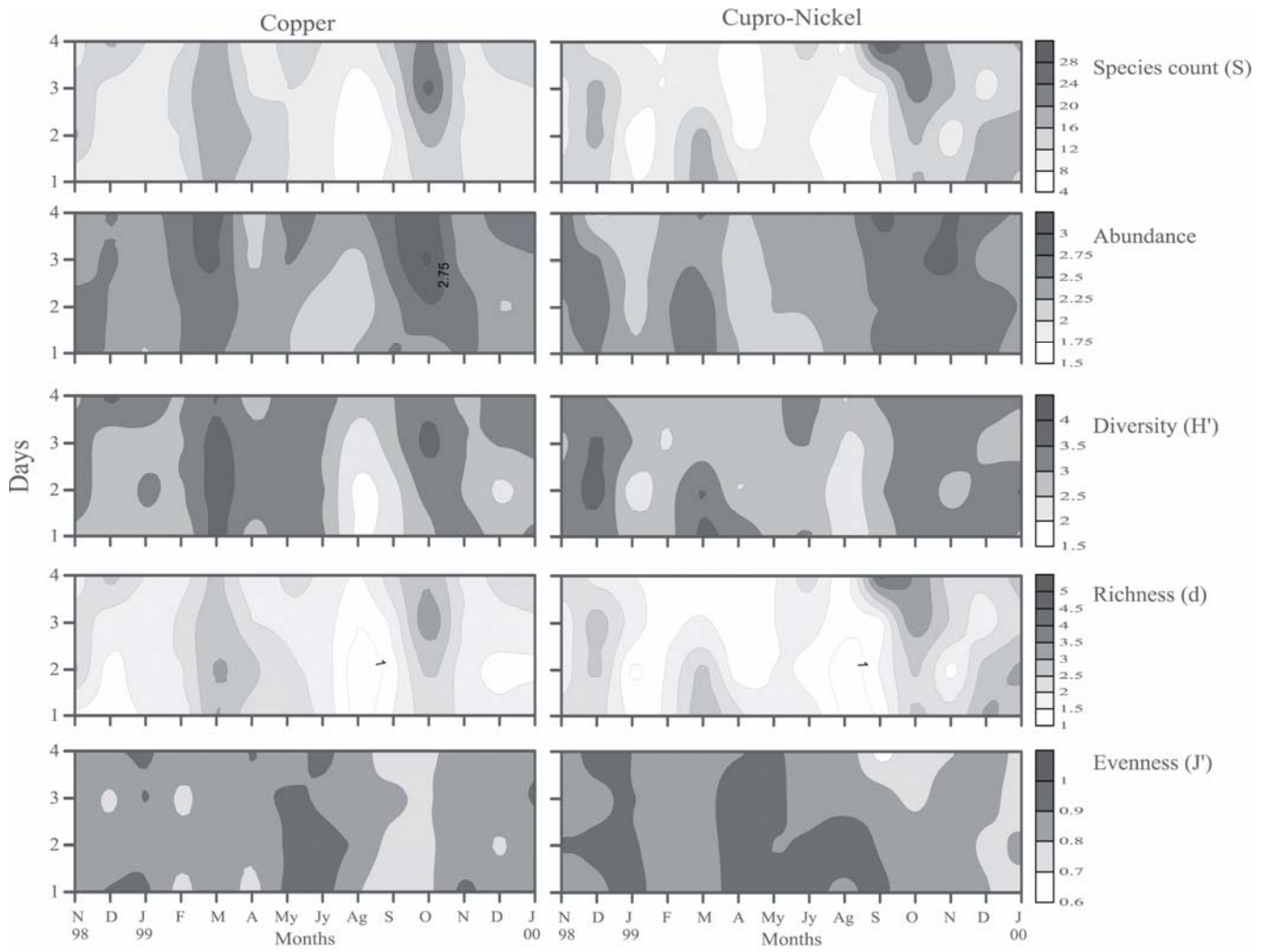


Fig. 2

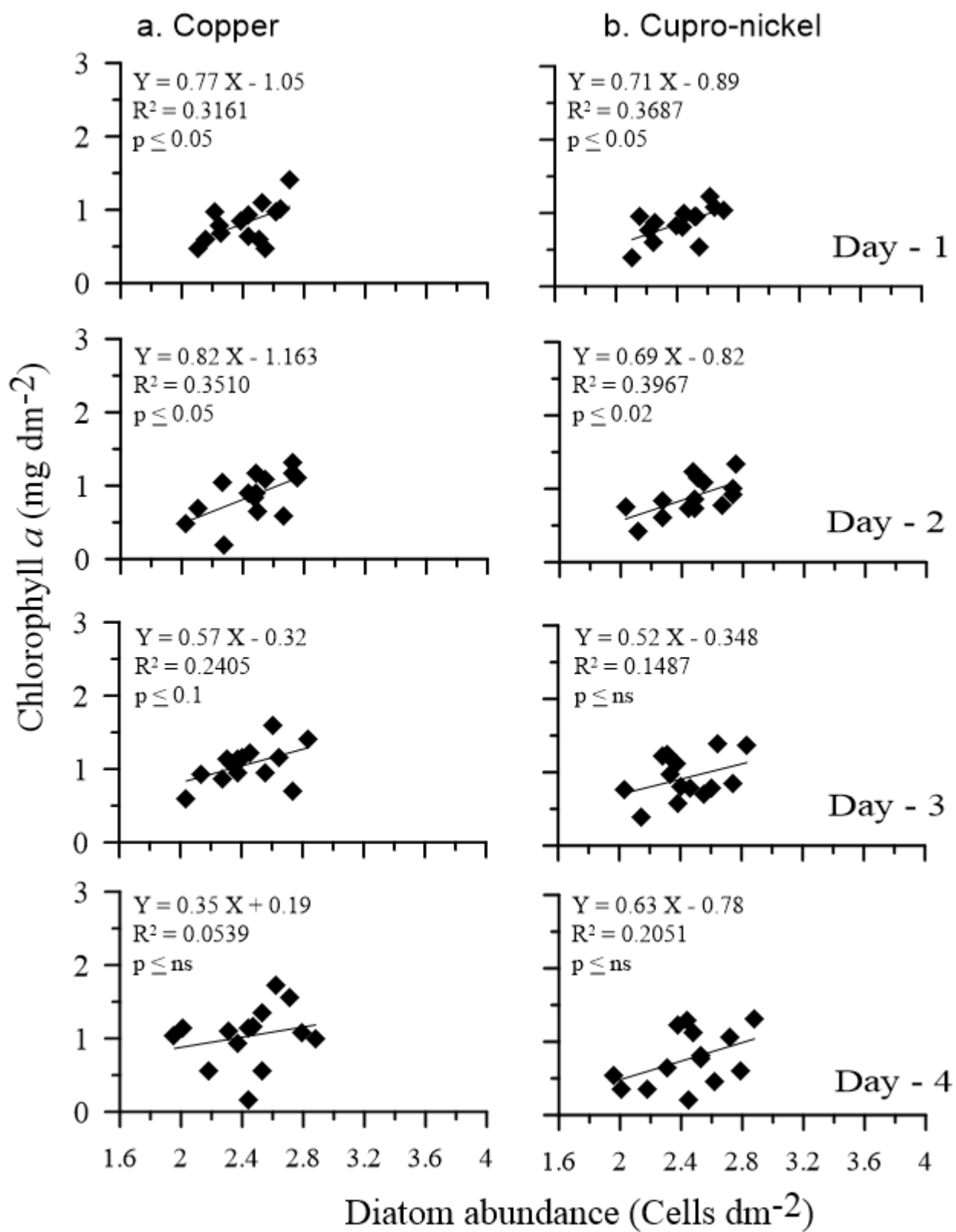


Fig. 3



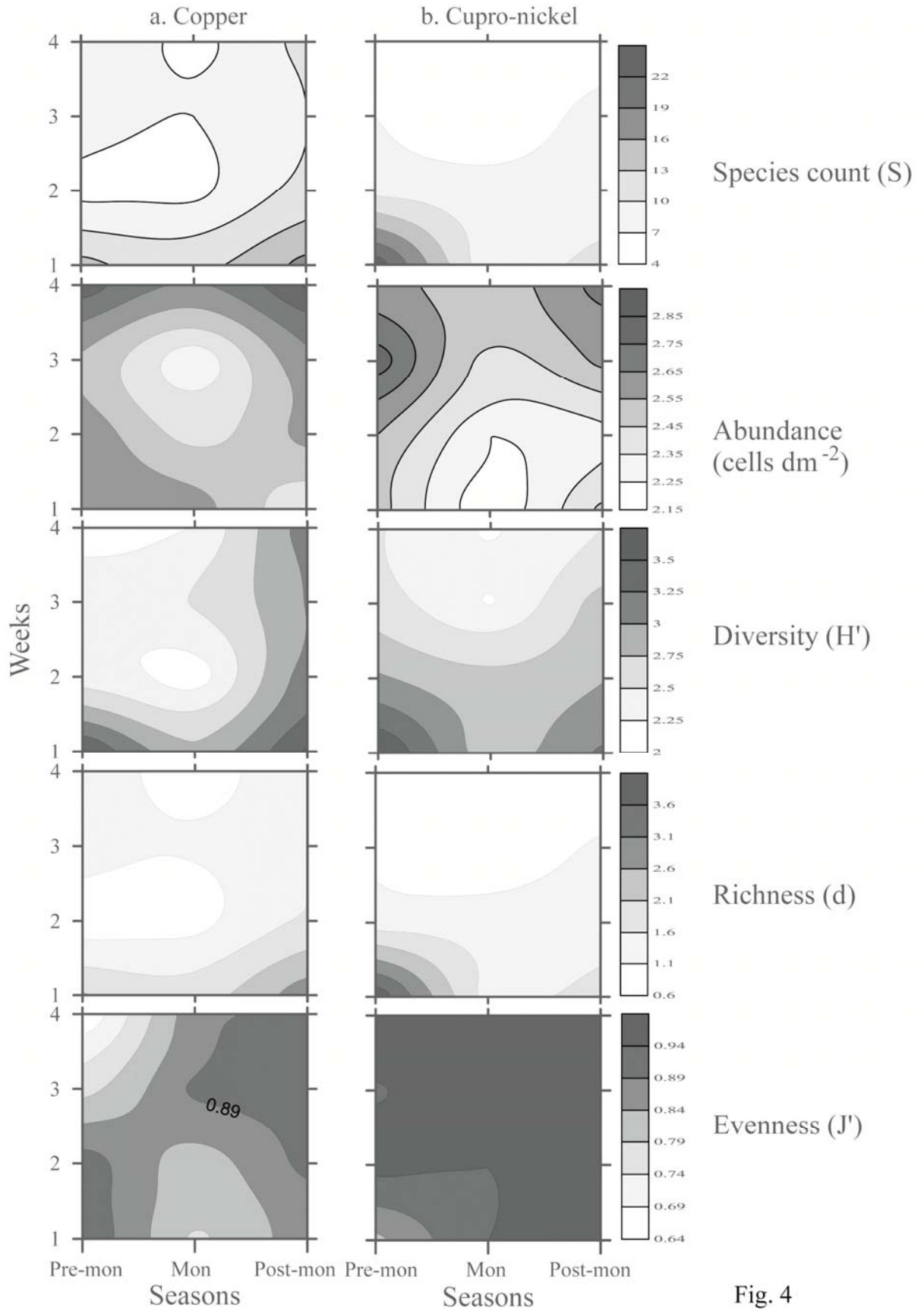


Fig. 4

## Legends to supplementary figures

**Supplementary fig. 1.** The ratio of pennate/centric diatoms in the microfilm developed over copper and cupro-nickel substrata exposed for different exposure periods i.e. days (a & b) to weeks (c & d).

**Supplementary fig. 2.** Dendograms of the biofilm diatom community developed on copper with respect to species for different submersion periods (1, 2, 3, and 4 d).

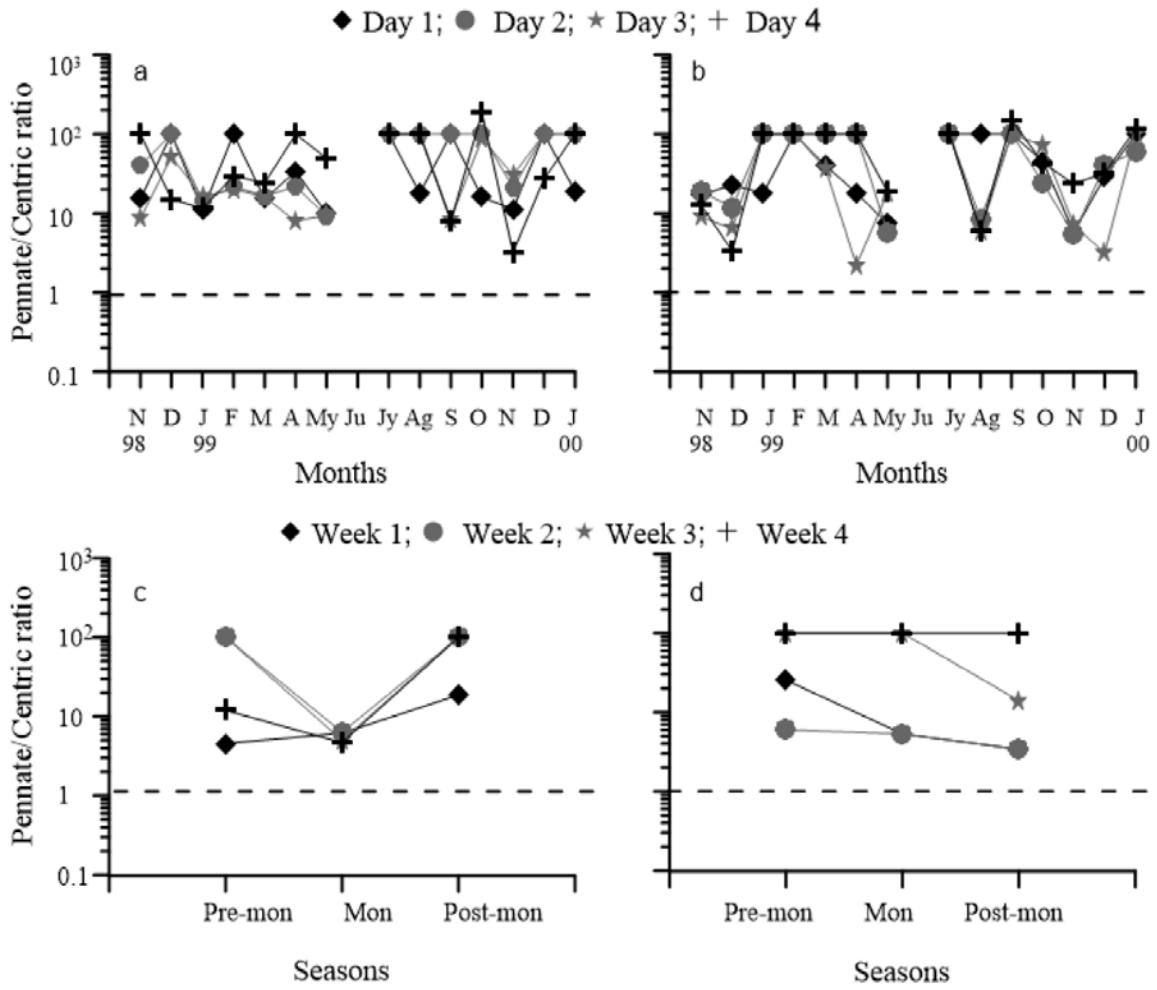
**Supplementary fig. 3.** Dendograms of the biofilm diatom community developed on cupro-nickel with respect to species for different exposure periods (1, 2, 3, and 4 d).

**Supplementary fig. 4.** Dendograms of the fouling diatom community developed on copper and cupro-nickel substrata with respect to season for different exposure periods (Week 1, Week 2, Week 3, and Week 4).

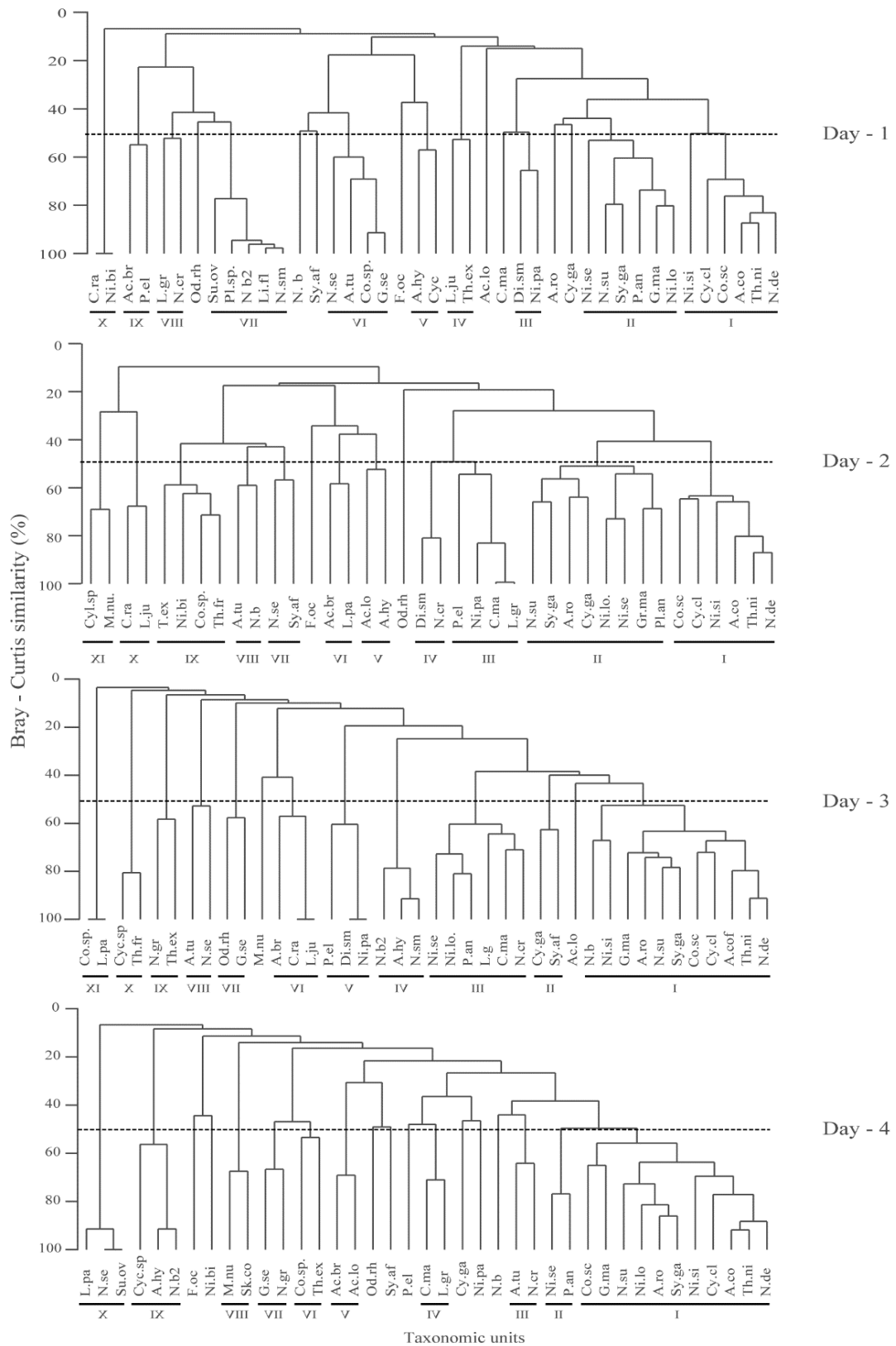
**Supplementary fig. 5.** Dendograms of the fouling diatom community developed on copper with respect to species for different exposure periods (Week 1, Week 2, Week 3, and Week 4).

**Supplementary fig. 6.** Dendograms of the fouling diatom community developed on cupro-nickel with respect to species for different exposure periods (Week 1, Week 2, Week 3, and Week 4).

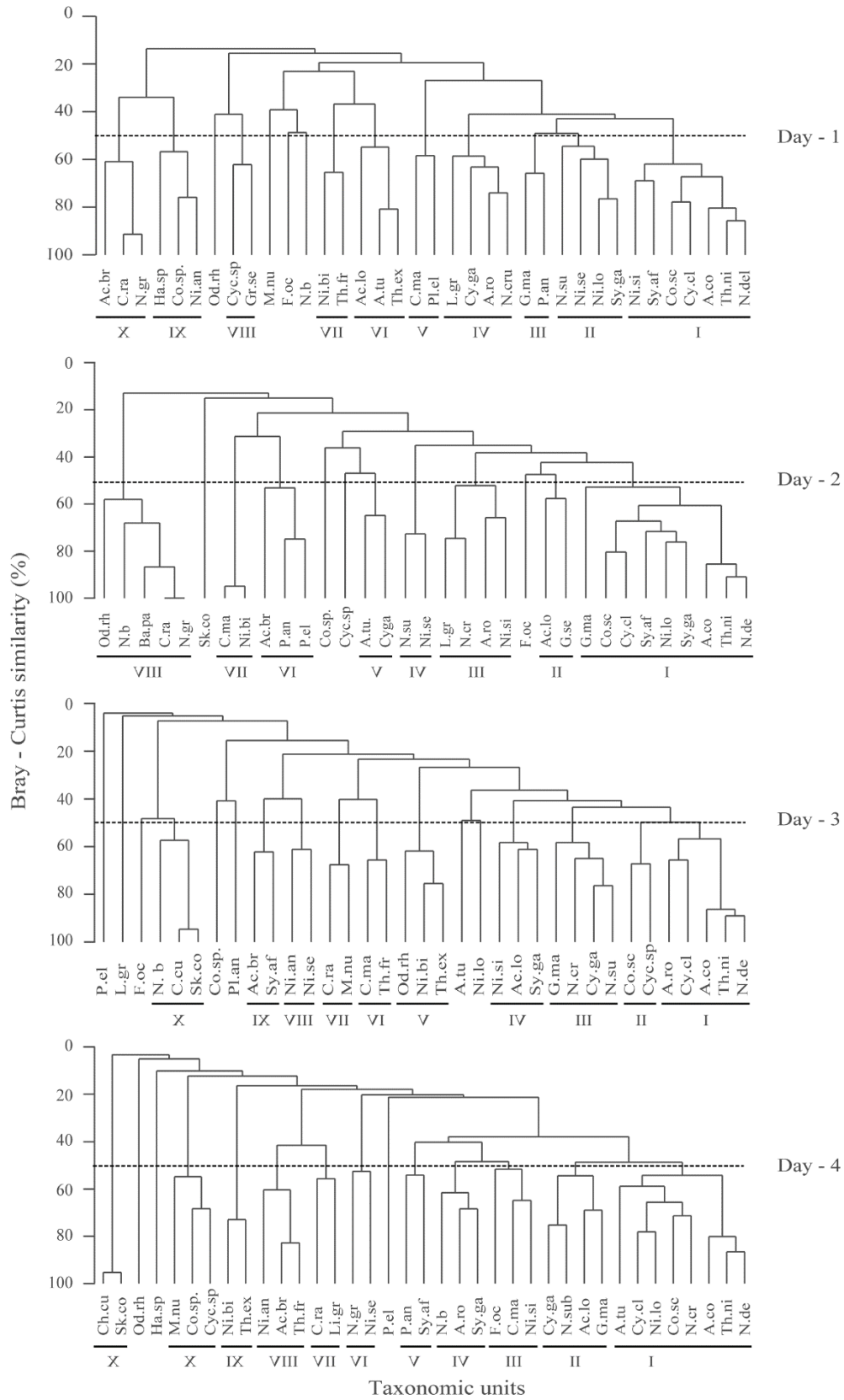
**Supplementary fig. 7.** Temporal and substratum variation in the abundance of microfilm diatoms from fiberglass (Fg), glass slide (Gl), copper (Cu) and cupro-nickel (Cu-Ni) at different exposure periods. *Note: fourth root transformed raw abundance data is used and the range of symbol height is 0 to 0.32 inch at data value 0 and 9.*



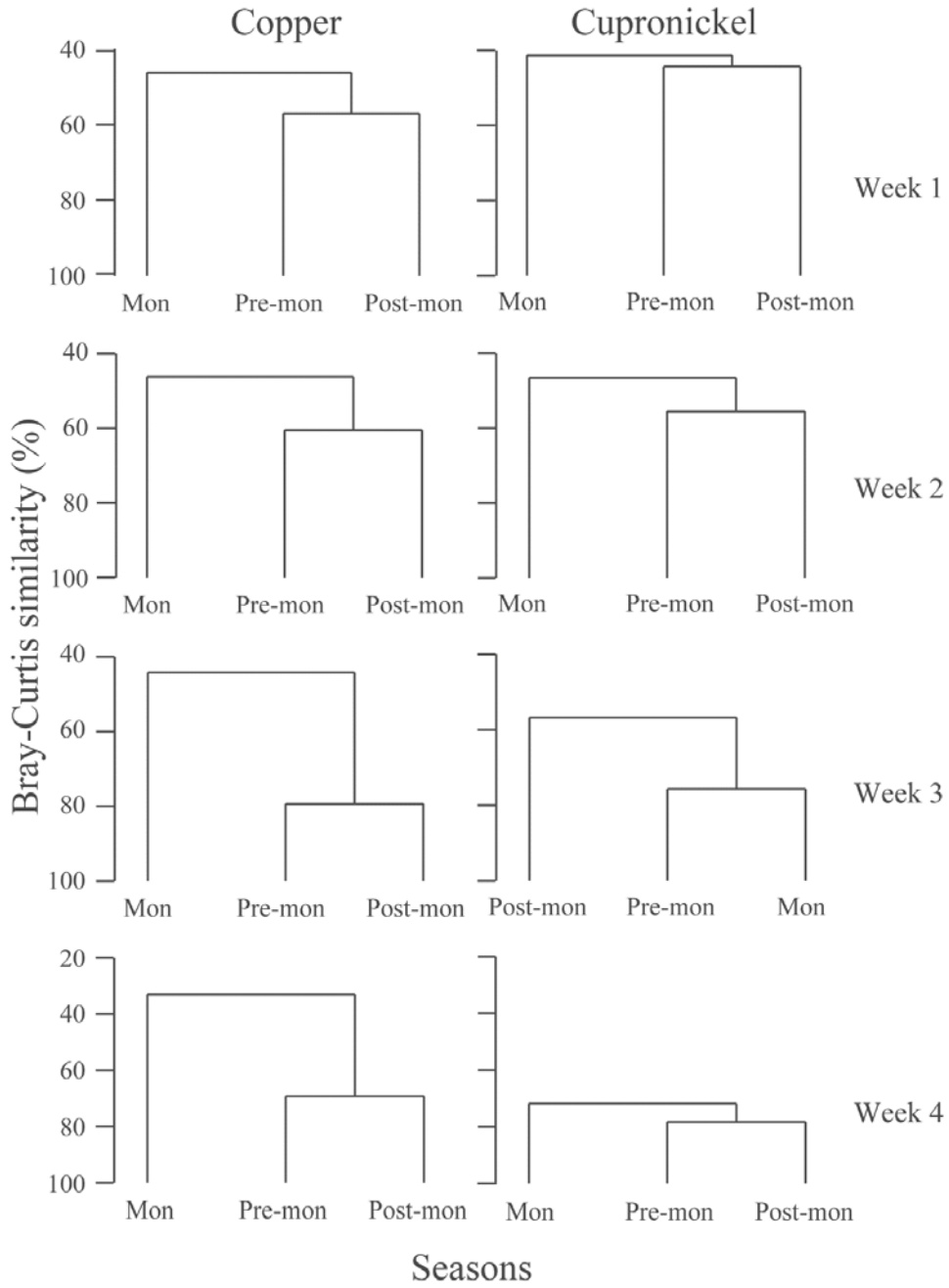
Supplementary Fig. 1



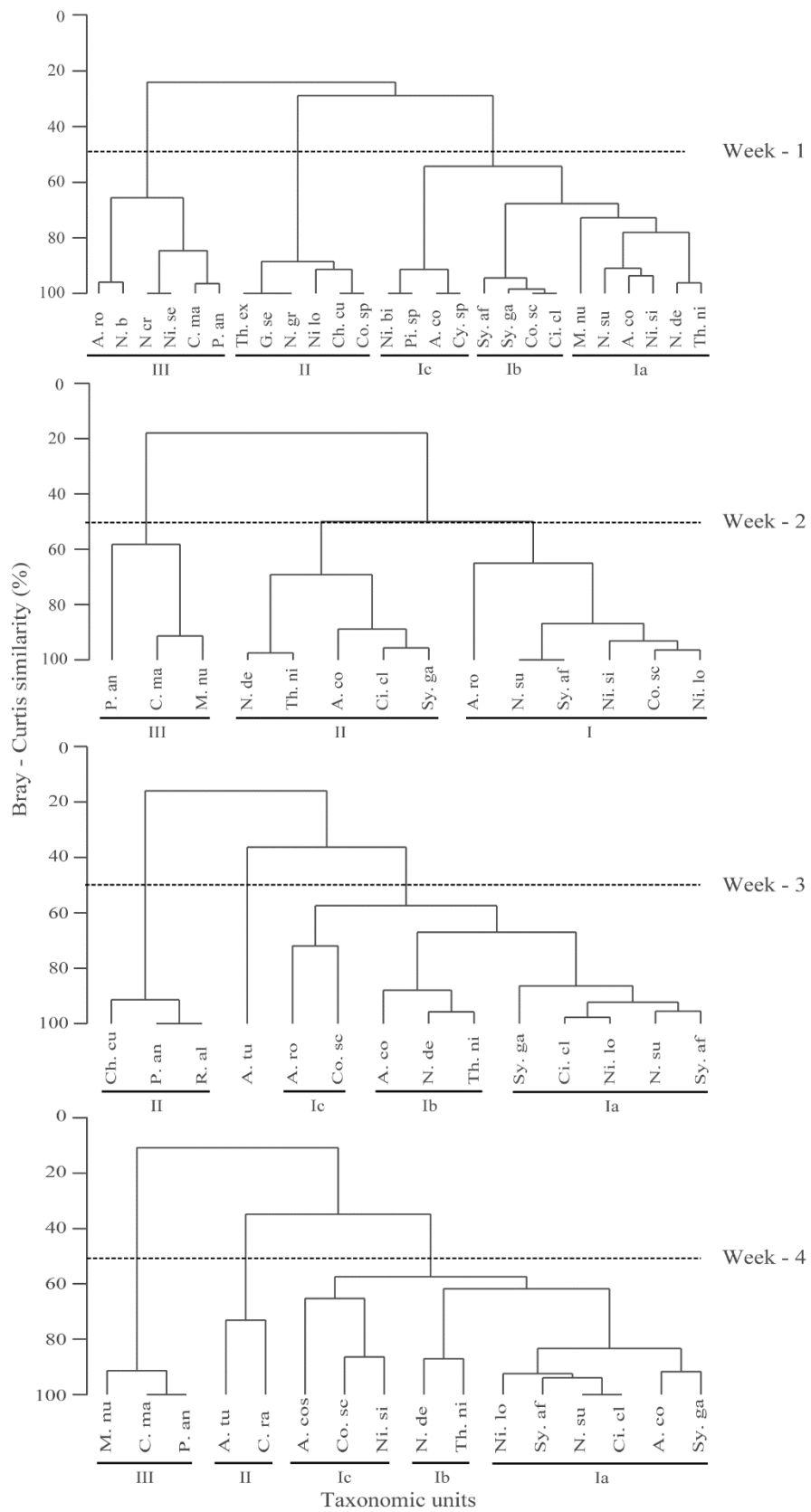
Supplementary Fig. 2



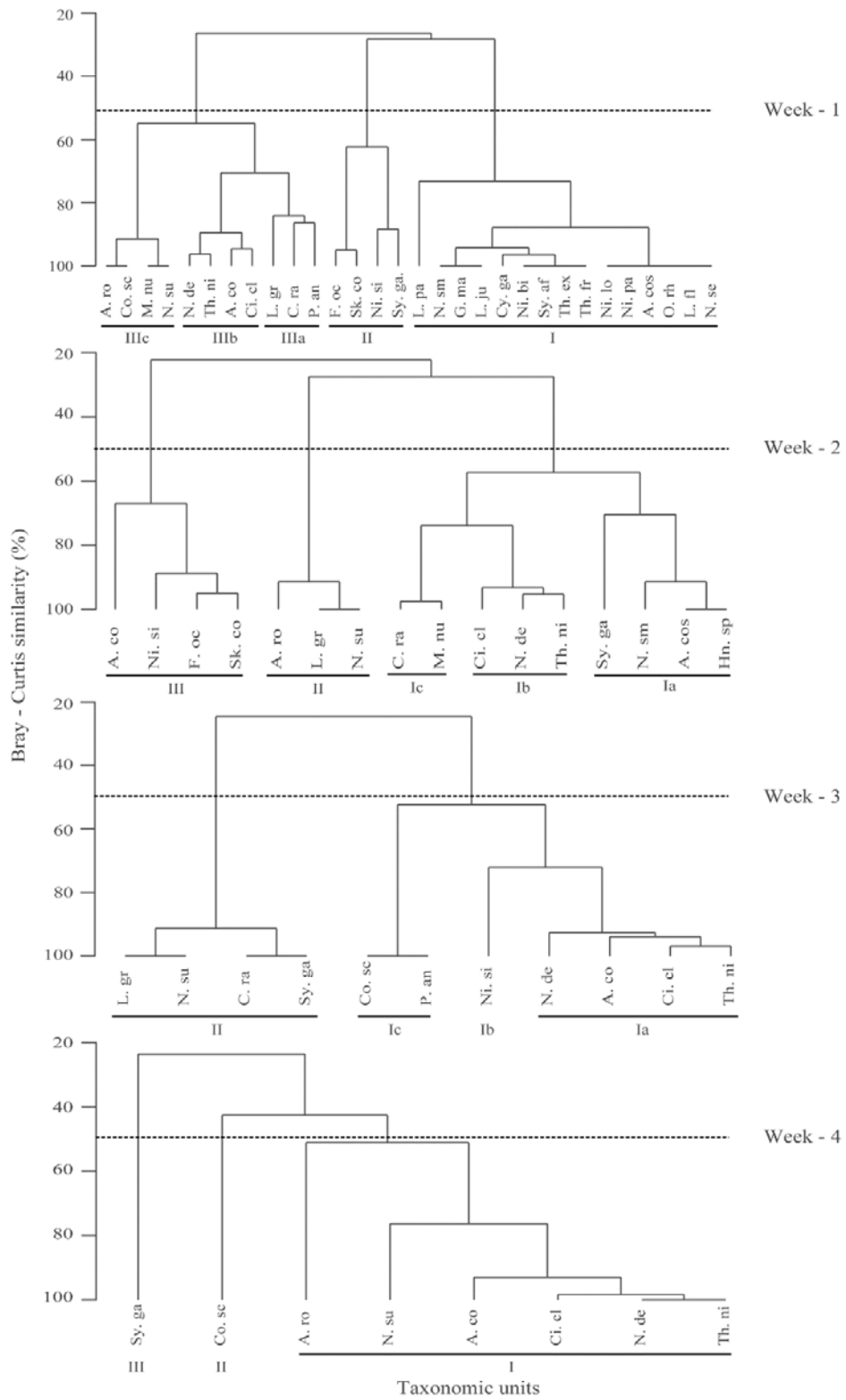
Supplementary Fig. 3



Supplementary Fig. 4

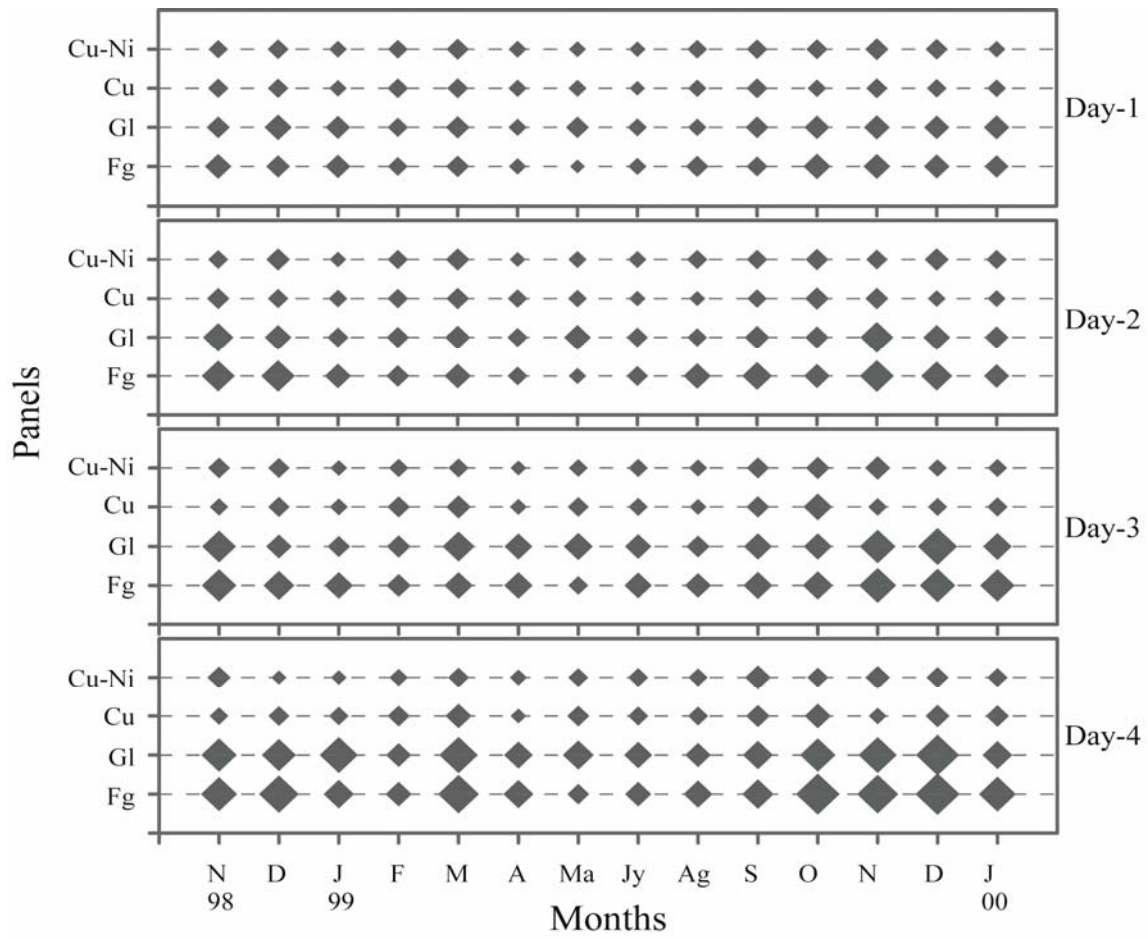


Supplementary Fig. 5



Supplementary Fig. 6





Supplementary Fig. 7

Supplementary table I. ANOVA to evaluate the temporal variation in the diatom count, abundance, diversity, richness and evenness on copper and cupro-nickel with respect to exposure (short term).

Source of variation	df	Count			Abundance			Diversity			Richness			Evenness		
		SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>
A: Substrata	1	9690.3	9690		0.06	0.06		545	545		247	247		43.6	43.6	
B: Months	14	814.33	58.17		16.5	1.18		5.39	0.38		17.8	1.27		0.11	0.01	
C: Days	3	31.413	10.47		1.34	0.45		0.11	0.04		0.48	0.16		0	0	
A*B	14	1101.5	78.68	4.28**	3.08	0.22	1.57 <sup>ns</sup>	7.89	0.56	4.20**	25.1	1.8	4.68**	0.19	0.01	4.60**
A*C	3	48.904	16.3	0.89 <sup>ns</sup>	0.53	0.18	1.27 <sup>ns</sup>	0.83	0.28	2.05 <sup>ns</sup>	0.9	0.3	0.79 <sup>ns</sup>	0.02	0.01	2.24 <sup>ns</sup>
B*C	42	260.4	6.2	0.34 <sup>ns</sup>	6.32	0.15	1.07 <sup>ns</sup>	1.53	0.04	0.27 <sup>ns</sup>	5.49	0.13	0.34 <sup>ns</sup>	0.05	0	0.41 <sup>ns</sup>
A*B*C	42	772.03	18.38		5.89	0.14		5.63	0.13		16.1	0.38		0.12	0	
Total	119	12719			33.8			566			313			44.1		

Supplementary table II. ANOVA to evaluate the temporal variation in the diatom count, abundance, diversity, species richness and evenness on non-toxic (fibreglass and glass) and toxic substratum (copper and cupro-nickel) with respect to exposure (short term).

Source of variation	df	Count			Abundance			Diversity			Richness			Evenness		
		SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>
A: Substrata	3	3154.0	1051.3		140.6	46.9		1.8	0.6		32.4	10.8		0.85	0.28	
B: Months	14	3754.6	268.2		60.6	4.3		26.7	1.9		70.8	5.1		0.90	0.06	
C: Days	3	345.0	115.0		49.3	16.4		0.1	0.0		0.8	0.3		0.10	0.03	
A*B	42	1590.1	37.9	2.76**	36.5	0.9	3.86**	16.3	0.4	4.19**	36.8	0.9	3.27**	0.76	0.02	5.95**
A*C	9	177.9	19.8	1.44 <sup>ns</sup>	30.8	3.4	5.21**	1.4	0.2	1.70 <sup>ns</sup>	2.5	0.3	1.03 <sup>ns</sup>	0.07	0.01	2.52*
B*C	42	430.0	10.2	0.75 <sup>ns</sup>	10.5	0.3	1.12 <sup>ns</sup>	4.4	0.1	1.14 <sup>ns</sup>	10.8	0.3	0.96 <sup>ns</sup>	0.12	0.00	0.94 <sup>ns</sup>
A*B*C	126	1730.1	13.7		28.3	0.2		11.7	0.1		33.7	0.3		0.38	0.00	
Total	239	11181.6			356.7			62.3			187.8			3.17		

Supplementary table III. ANOVA to evaluate the temporal variation in the diatom count, abundance, diversity, richness and evenness on copper and cupro-nickel substratum with respect to exposure (long term).

Source of variation	df	Count			Abundance			Diversity			Richness			Evenness		
		SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>	SS	MS	<i>F<sub>s</sub></i>
A: Substrata	1	1.5	1.5		0.1	0.1		0.01	0.01		0.02	0.02		0.0	0.01	
B: Seasons	2	1718.4	859.2		352.4	176.2		147.2	73.6		40.6	20.31		15.2	7.60	
C: Weeks	3	42.6	14.2		0.27	0.09		0.5	0.17		1.45	0.48		0.00	0.00	
A*B	2	35.9	17.9	1.53 <sup>ns</sup>	0.33	0.16	0.88 <sup>ns</sup>	0.5	0.23	3.59*	0.98	0.49	1.25 <sup>ns</sup>	0.04	0.02	2.47 <sup>ns</sup>
A*C	3	3.7	1.2	0.10 <sup>ns</sup>	0.14	0.05	0.25 <sup>ns</sup>	0.1	0.02	0.29 <sup>ns</sup>	0.12	0.04	0.11 <sup>ns</sup>	0.00	0.00	0.09 <sup>ns</sup>
B*C	6	206.5	34.4	2.93*	1.36	0.23	1.22 <sup>ns</sup>	2.3	0.39	6.12**	6.8	1.13	2.91*	0.02	0.00	0.46 <sup>ns</sup>
A*B*C	6	70.5	11.7		1.11	0.18		0.4	0.06		2.3	0.39		0.05	0.01	
Total	23	2079.0			355.6			151.0			52.3			15.3		